

**Design and in-house validation of a portable system for the determination
of free acidity in virgin olive oil**

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18 **ABSTRACT**

19 Nutritional and healthy values are well known properties of virgin olive oil (VOO). The product
20 quality, in terms of belonging to a specific quality grade (extra virgin, virgin, lampante), is defined
21 by a set of chemical-physical and sensory measurements. According to the official regulation of the
22 European Union (EU Reg. 1348/2013) the free acidity is the first parameter that has to be
23 determined by analysts; it gives information about the quality of the olives used to produce the
24 VOO as well as the hydrolytic state of VOO just produced and stored. The official procedure is
25 based on an acid-base titration that needs to be carried out in a chemical laboratory.

26 In this paper a portable battery-operated electronic system to measure olive oil free acidity is
27 presented: the system can be used for quick “in situ” tests in a production environment (olive oil
28 mills or packaging centers) by people without particular training. The working principles of the
29 system is based on the creation of an emulsion between oil and a hydroalcoholic solution: the free
30 acidity is estimated on the value of the emulsion electrical conductance.

31 This new system has been calibrated and in-house validated showing good results in terms of limit
32 of detection and quantification, precision and accuracy, beyond a good correlation with free acidity
33 data obtained applying the official method ($R^2_{\text{adj}} = 0.97$).

34

35 **Keywords:** portable system; free acidity; virgin olive oil; impedance spectroscopy; in-house
36 validation; electrical conductance.

37

38 **Abbreviations**

39 **EIS:** electrical impedance spectroscopy, **EVOO:** extra virgin olive oil, **FA:** free acidity, **LOD:**
40 limit of detection, **LOO:** lampante olive oil, **LOQ:** limit of quantification, **RSD:** relative standard
41 deviation, **TAGs:** triglycerides, **VOO:** virgin olive oil.

1. Introduction

Virgin Olive Oil (VOO) is obtained from olives (the fruits of *Olea europaea L.*) applying only a mechanical-physical extraction process and represents a product highly appreciated for its beneficial effects on human health, mainly due to a high content of oleic acid and minor components such as phytosterols, carotenoids, tocopherols and hydrophilic phenols (Bendini et al., 2007). The European Commission Implementing Regulation 1348/2013 (EU Reg. No 1348/2013) defines a decision tree for verifying whether a VOO is consistent with the category declared, and the quality criteria that have to be checked by analysts are: free acidity (FA), peroxide value, specific extinctions in UV, sensory characteristics and ethyl esters of fatty acids. The first quality parameter in the above cited decision tree is, therefore, the determination of the free acidity of the oil sample; this is defined as the amount of free fatty acids, no longer linked to their parent triglyceride molecules (TAGs), and measured as percentage of oleic acid. Specifically, the top-quality product, Extra Virgin Olive Oil (EVOO), features a maximum FA of 0.8 g oleic acid/100 g oil, then the VOO features a maximum FA value of 2.0 g oleic acid/100 g oil and, finally, the Lampante Olive Oil (LOO), that is not suitable for the commercialization as it is, is characterized by a FA higher than 2.0 g oleic acid/100 g oil.

This parameter is especially affected by the quality of the olives used to produce the oil since free fatty acids arise from the separation of fatty acids from TAGs because of the action of enzymes, further stimulated by light, water, and heat. Thus, FA is an indicator of how fresh and how well handled the olives were before being milled (Tena, Wang, Aparicio-Ruiz, García-González, & Aparicio, 2015). To confirm this, different studies reported that geographic and environmental factors (Bustan et al., 2014) and the application of specific technological processes (such as filtration or a cooling treatment of olive paste) (Veneziani et al., 2018 a-b) do not affect significantly this parameter.

The official procedure to measure the oil free acidity is defined by the European regulations (EEC Reg. 2568/1991 and following amendments) and consists of an acid-base titration that, albeit simple and quick, must be carried out in a laboratory by trained personnel essentially due to the need of specific solvents and lab equipment.

However, in addition to official methods for the quality control of virgin olive oils, there is a strong need for simple, rapid and environmental friendly techniques, also suitable for on-site quality control even for new clients, who are “non-professional analytical skilled” end users (Inajeros-García, Gómez-Alonso, Fregapane, & Salvador, 2013; Valli et al., 2016). This represents an important issue in particular for the small oil mills and packaging centers that cannot afford the cost of external laboratory analysis.

76 The possibility of simple, quick and in-situ analysis for the food quality control (often implemented
77 in the form of portable electronic systems) has been widely researched in the last years (Oujji et al.,
78 2014; Grossi, Di Lecce, Arru, Gallina Toschi, & Riccò, 2015; Sture, Ruud Oye, Skavhaug, &
79 Mathiassen, 2016; Arsalane et al., 2018). Concerning the olive oil free acidity determination,
80 different innovative techniques have been presented in literature (Valli et al., 2016). One approach
81 is based on Near-Infrared (NIR) spectroscopy, allowing to estimate acidity by optical spectroscopy
82 analysis of the transmission spectra in the wavenumber range 4541 to 11726 cm^{-1} (Armenta,
83 Garrigues, & de la Guardia, 2007). This solution has the advantage that the measurement can be
84 carried out on the oil sample without any reagent, but it needs expensive instrumentation (optical
85 spectrophotometer) and requires frequent re-calibration (different calibrations must be carried out
86 depending on olives varieties or geographical origin etc.). A pH-metric procedure to measure oil
87 acidity has been also proposed (Tur'yan, Berezin, Kuselman, & Shenhar, 1996): the oil sample is
88 extracted with a chemical solvent (0.20 M triethanolamine in a 1:1 solution of water and
89 isopropanol) and the pH measured. This technique is reported to be accurate, but the pH probe
90 needs to be used with care and frequent cleaning and re-calibration are needed. Moreover, the used
91 chemicals add costs for disposal after use.

92 The aim of this work is to present a portable electronic system to measure the free acidity in olive
93 oil samples: the instrument is battery-operated and can be used for quick “in-situ” measurements in
94 the oil production or bottling site. The working principle is based on the estimation of the oil FA
95 from the electrical conductance measured by Electrical Impedance Spectroscopy (EIS) of the oil
96 emulsion with a hydroalcoholic solution. EIS is used in a wide range of applications (Grossi, &
97 Riccò, 2017a), such as to estimate the ripening degree of fruits (Harker, & Maindonald, 1994), to
98 characterize plant tissues (Lin, Chen, & Chen, 2012; Ben Hamed, Zorrig, & Hichem Hamzaoui,
99 2016), to characterize and to detect the freezing end point of ice cream mixes (Grossi, Lanzoni,
100 Lazzarini, & Riccò, 2012a), to estimate the water content in extra virgin olive oil (Ragni et al.,
101 2013), to investigate the corrosion of metal surfaces exposed to acid electrolytes (Ansari, Quraishi,
102 & Singh, 2015; Hussin, Rahim, Nasir, Ibrahim, & Brosse, 2016), to analyse human body
103 composition (Khalil, Mohktar, & Ibrahim, 2014; Bera, 2014), to detect the end-point in a titration
104 assay (Grossi, & Riccò, 2017b).

105 The system described in this paper represents a considerable improvement over previous studies
106 (Grossi, Di Lecce, Gallina Toschi, & Riccò, 2014a-b) since it has been completely re-designed with
107 focus on low-cost electronics and small dimensions. To make the system completely portable and
108 reliable for on-site measurements, a temperature sensor has been included to compensate for
109 conductance variations linked to changes in environmental temperature. Moreover, the presented

instrument has been in-house validated evaluating its performances in terms of correlation between the concentration of the analyte and the instrument response, limit of detection (LOD) and quantification (LOQ), precision and accuracy. The validation step, in fact, is one of the measures universally recognized as a necessary part of a comprehensive system of quality assurance in analytical chemistry and is an essential component of the measures that a laboratory should implement to allow it to produce reliable analytical data (Thompson, Ellison, & Wood, 2002).

2. Material and methods

2.1 Reagents and chemicals

Diethyl ether (ACS reagent, purity $\geq 99.8\%$), ethanol (ACS reagent, purity $\geq 96\%$) and phenolphthalein solution (indicator, 1% in ethanol) were supplied from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Sodium hydroxide 0.1 mol/L (N/10) was purchased from Carlo Erba Reagents S.r.l. (Milan, Italy).

Distilled water was produced by Elix Essential system (Millipore, Molsheim, France). Oleic acid (CAS Number 112-80-1, assay 90%) used as standard for building the calibration curve was supplied from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

2.2 Samples

In order to calibrate the system, 5 samples of refined sunflower oil (with free acidity and peroxide value under the limits for vegetables oils according to CODEX STAN 210-1999) added with oleic acid were prepared. To cover the range of free acidity of the different quality grades (EVOO, VOO, LOO) different concentrations of oleic acid (from 0% to 3.75%) were added (Table 1).

The in-house validation study was carried out on a set of 30 olive oil (numerically coded from 1 to 30) with a different quality grade: 20 EVOOs, 7 VOOs and 3 LOOs. An aliquot of 50 mL for each sample was stored in PET dark bottles at 11-12 °C before the analysis.

2.3 Determination of free acidity

2.3.1 Official method

The determination of FA by titration was applied according to the EU Reg. 2016/1227. An aliquot of the oil sample (the weight depends on the presumed free acidity) was dissolved in 100 mL of a mixture of diethyl ether and ethanol (1:1 v/v, previously neutralized) and the free fatty acids present were neutralized using sodium hydroxide solution (0.1 M). Phenolphthalein was used as indicator.

2.3.2 Portable system

The working principle of the portable system (Figure 1) is based on the measure of the electrical conductance of an emulsion between a hydroalcoholic solution and the oil sample. In presence of the hydroalcoholic solution, the free fatty acid molecules RCOOH , where R is the hydrocarbon

144 chain, dissociate in the ionic compounds H_3O^+ and RCO_2^- that contribute to the increase of the
145 emulsion electrical conductance. Consequently, the higher the free fatty acid molecules
146 concentration, the higher ions concentration and the higher the electrical conductance.

147 A 50 mL round bottom polypropylene tube (Falcon) modified to feature a couple of cap-shaped
148 stainless-steel electrodes (6 mm in diameter, spaced by 12 mm one from the other) required for the
149 electrical characterization is used as sensor to realize the measurement.

150 The first step to realize the measurement is a “reagent test” to check the electrical properties
151 (electrical conductance) of the hydro-alcoholic solution in order to avoid an overestimation of the
152 oil free acidity linked to the conductance of the solution. For this purpose, the tube (sensor) is filled
153 with 9 mL of ethanol and 6 mL of distilled water and its conductance is measured. If the reagent
154 passes the test and is suitable for the measure (conductance not higher than $0.6 \mu\text{S}$), 1 mL of the oil
155 sample to be tested is added and the obtained mixture is manually shaken vigorously for about 20
156 seconds to create an emulsion, whose conductance is measured to estimate the oil acidity. A video
157 showing the procedure to realize the analysis is available in the online version of the paper.

158 The instrument is also equipped with a temperature sensor to measure the room environmental
159 temperature during the analysis. The emulsion electrical conductance and the temperature are
160 measured and these values are used to calculate the emulsion conductance at the calibration
161 temperature (23.5°C). Then the free acidity of the sample is estimated using the calibration
162 function stored inside the microcontroller non-volatile memory.

163 **2.4 In-house method validation**

164 The following parameters were evaluated in order to ensure the method quality: limit of detection
165 (LOD) and quantification (LOQ), intra-day and inter-day precision and accuracy.

166 Before the application of the portable system on olive oil samples and in order to verify the
167 correlation between the concentration of the analyte and the response of the instrument, a
168 calibration using samples of refined sunflower oil added with oleic acid previously described (see
169 paragraph 2.2) was carried out. All the samples were analyzed in triplicate by both the official
170 method to determine the reference value of FA, and by the portable system to measure the
171 conductance value. Then, the values were plotted in order to study the correlation between the
172 electrical conductance and the free acidity of the samples.

173 The limit of detection (LOD) and quantification (LOQ) were evaluated considering the linear
174 relation between the conductance measured by the portable system and the square root of FA. The
175 following formulas were applied:

$$176 \quad LOD = 3\sigma/m \quad (1)$$

$$177 \quad LOQ = 10\sigma/m \quad (2)$$

178 where σ is the standard deviation of the FA square root measured for the refined sunflower oil
179 without addition of oleic acid (sample A) and m is the slope of the curve.

180 To evaluate the inter-day precision of the method, two virgin olive oil samples for each type of
181 quality grade (samples EVOO_2 and EVOO_19 for EVOOs, samples VOO_1 and VOO_7 for
182 VOOs and samples LOO_1 and LOO_3 for LOOs) were analyzed in triplicate for three different
183 days and values were statistically evaluated by the Student's Test ($p < 0.05$).

184 Accuracy and intra-day precision were studied analyzing the set of 30 olive oils with a different
185 quality grade. Precision were expressed in terms of RSD (Relative Standard Deviation) measured
186 for the free acidity values obtained with the portable system. For the accuracy, all the samples were
187 also analyzed following the official method (acid-base titration) and the comparison between the
188 values obtained by the two methods was performed (two-tailed paired t-test with $p < 0.05$).

189

190 **3. Results and discussion**

191 **3.1 Design of the portable system**

192 A portable battery-operated electronic system for in-situ measurements of olive oil free acidity has
193 been built to allow olive oil quality assurance directly in the oil mills or packaging centers.

194 The dimensions of the instrument are 11 x 15 x 5 cm and some pictures are presented in Figure 2a.
195 The instrument can be powered by USB port or using batteries (3 AAA alkaline batteries 1.5 V).
196 The system is composed of an electronic board designed ad-hoc that performs all the operations to
197 measure the free acidity, a 2 rows 16 columns LCD screen to output the measure results, four
198 buttons for user interaction and the sensor previously described (see paragraph 2.3.2). The
199 electronic board is based on the microcontroller STM32L152RCT6A and its schematic is presented
200 in Figure 2b.

201 To determine the FA of an oil sample the electrical conductance of the emulsion is measured by
202 Electrical Impedance Spectroscopy (EIS). In the proposed approach the sample under investigation
203 is stimulated with a 1 V 200 Hz sine-wave voltage signal $V_{in}(t)$ generated by the built-in 12-bits
204 DAC inside the microcontroller and applied to the sensor electrodes.

205 The current drawn by the sensor is converted to a sine-wave voltage $V_{out}(t)$ by means of a current-
206 to-voltage converter. Given

$$207 \quad V_{in}(t) = V_{M,in} \times \sin(2\pi ft) \quad (3)$$

208 it is

$$209 \quad V_{out}(t) = V_{M,out} \times \sin(2\pi ft + \varphi) \quad (4)$$

210 where $V_{M,in}$ and $V_{M,out}$ are the amplitude of the corresponding signals, φ is the phase difference
211 between the current through the sample and $V_{in}(t)$, while f is the frequency of the test signal (200
212 Hz).

213 Both $V_{in}(t)$ and $V_{out}(t)$ are acquired by the built-in 12-bits ADC inside the microcontroller using a
214 sampling frequency of 50 kHz and the sine-wave parameters are calculated using the algorithm
215 previously presented by Grossi et al. (Grossi, Lanzoni, Lazzarini, & Riccò, 2012b).

216 The emulsion in direct contact with the electrodes can be modelled as the parallel of an electrical
217 conductance (accounting for the conductance of the emulsion) and a capacitance (accounting for the
218 emulsion dielectric properties): while the emulsion conductance (that dominates at low frequency)
219 is affected by the sample acidity due to the variation of the ions concentration, the dielectric
220 properties are almost independent.

221 The electrical conductance (G_m) is thus calculated as:

$$222 \quad G_m = \frac{I}{R_F} \times \frac{V_{M,out}}{V_{M,in}} \times \cos(\varphi) \quad (5)$$

223 where R_F is the feedback resistance (470 k Ω) of the current-to-voltage converter.

224 However, the relation between G_m and the free acidity is non-linear and it can be modelled with the
225 function:

$$226 \quad G_m = \alpha + \beta \times \sqrt{FA} \quad (6)$$

227 where α, β are empirical parameters that must be determined by a suitable calibration procedure and
228 are also function of the calibration temperature.

229 Then, the sample free acidity can be estimated from the measured electrical conductance of the
230 emulsion with the following formula:

$$231 \quad FA = ((G_m - \alpha) / \beta)^2 \quad (7)$$

232 Since the system must be operated “in the field” and the environmental temperature is not a
233 parameter under control, the environmental temperature (T) is measured by means of a MCP9700A
234 analog temperature sensor integrated on the electronic board: the output voltage (that has a
235 sensitivity of 10 mV/°C) is acquired by a channel of the microcontroller ADC and converted to the
236 temperature value.

237 The system works as follows: the emulsion electrical conductance registered at a certain
238 temperature ($G_{m,T}$) and the temperature (T) are measured and these values are used to calculate the
239 emulsion conductance at the calibration temperature ($G_{m,Tcalib}$). Then the free acidity of the oil
240 sample is estimated from the calculated value of $G_{m,Tcalib}$ using the calibration function stored inside
241 the microcontroller non-volatile memory.

A more detailed description of the system by an electronical and mathematical point of view is presented in Appendix A, while a discussion on the influence of the environmental temperature on the measured conductance and the method used for compensation is presented in Appendix B.

3.2 In-house method validation

Before being used with real olive oil samples, the portable instrument has been calibrated. For this purpose, all the samples of refined sunflower oil added with oleic acid (samples A-E) were analyzed by both the official method to determine the reference value of free acidity and by the portable system to measure the conductance value. The data obtained are shown in Table 1. Then the values were plotted (Figure 3): in all cases the electrical conductance measured for the samples increases with its free acidity with a non-linear relation, confirming what previously presented (Grossi, Di Lecce, Gallina Toschi, & Riccò, 2014b). Starting from these data and applying a nonlinear regression, the equation of the calibration curve was defined as follow:

$$FA = \left(\frac{G_{m,23.5^{\circ}C} + 0.0678}{2.7877} \right)^2 \quad (8)$$

where $G_{m,23.5^{\circ}C}$ is the emulsion electrical conductance at the calibration temperature of 23.5 °C. It allows to obtain, directly on the display of the portable system, the free acidity value of the tested sample estimated starting from its electrical properties.

Subsequently, some parameters for the in-house validation of the instrument were evaluated in order to check its performances and applicability on real olive oil samples.

LOD and LOQ were measured considering the FA of the refined sunflower oil and the slope of the linear correlation between the free acidity square root and the conductance. The values obtained were 0.02% and 0.06% of oleic acid for LOD and LOQ respectively.

Next, a set of 30 olive oil samples with a different quality grade (20 Extra Virgin Olive Oils – EVOO; 7 Virgin Olive Oils – VOO; 3 Lampante Olive Oils – LOO) were analyzed in triplicate applying this new analytical approach in order to study its accuracy and intra-day precision (expressed in terms of RSD).

The portable instrument showed good precision results since all the obtained values were under the 15%. All the samples were also analyzed following the official method (acid-base titration) and the comparison between the values obtained by the two methods was performed to measure the accuracy of the system. The differences between the two series of results (official method vs portable system) were evaluated by using the two-tailed paired t-test ($p < 0.05$). The two approaches did not give statistically differences for the mean values ($t < t_{critical}$). Moreover, the regression between the two series of values provided a coefficient R^2_{adj} of 0.97 (Figure 4) in agreement with data previously presented by Grossi et al. (Grossi, Di Lecce, Gallina Toschi, &

275 Riccò, 2014). Considering the commercial categories of the samples analyzed, all of them, with the
276 only exception of the sample VOO_7, were classified in the same way by both approaches. Finally,
277 considering the inter-day precision of the instrument, no significative differences (Student's Test,
278 $p < 0.05$) were found among the results obtained for each analyzed sample in the three different
279 days.

280

281 **4. Conclusions**

282 The design and in-house validation of a portable battery-operated electronic system suitable for in-
283 situ measurements of olive oil free acidity has been presented. The system is built with low cost
284 electronics and embeds a temperature sensor to compensate variations of the measured electrical
285 parameters with the environmental temperature, thus making it suitable for on-site free acidity
286 measurements outside a laboratory. Its working principle is based on the estimation of the olive oil
287 FA from the measure of the conductance of an emulsion between a hydro-alcoholic solution and the
288 sample to be tested. When the free fatty acids present in the sample comes in contact with the
289 hydro-alcoholic solution, a dissociation occurs, leading to the formation of ions that produce an
290 increase of the measured electrical conductance.

291 The system has been calibrated and in-house validated. The data obtained showed good
292 performances of the instrument in terms of LOD and LOQ, intra-day and inter-day precision.
293 Moreover, it showed a good correlation ($R^2_{\text{adj}} = 0.97$) with the FA evaluated applying the official
294 method demonstrating a satisfactory accuracy.

295 The procedure to realize the analysis is very quick and easy. This make the system suitable also for
296 people without specific training. The application of this analytical device is addressed, in particular,
297 to the estimation of free acidity of just produced virgin olive oils in oil mills. A next study will be
298 focused on the evaluation of the performance of this portable system when applied to bottles stored
299 for several months in dark/light conditions simulating possible different commercial
300 conservation/exposure on shelves of groceries. This study will give indications regarding the
301 maximum oxidation state of olive oil sample tolerable to use this portable system as accurate and
302 reliable.

303

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310

311 **Declaration of interest**

312 None.

313

314 **References**

315

316 Ansari, K. R., Quraishi, M. A., Singh, A. (2015). Pyridine derivatives as corrosion inhibitors for
317 N80 steel in 15% HCl: electrochemical, surface and quantum chemical studies. *Measurement*, 76,
318 136-147.

319

320 Armenta, S., Garrigues, S., de la Guardia, M. (2007). Determination of edible oil parameters by
321 near infrared spectrometry. *Analytica Chimica Acta*, 596, 330-337.

322

323 Arsalane, A., El Barbri, N., Tabyaoui, A., Klilou, A., Rhofir, K., Halimi, A. (2018). An embedded
324 system based on DSP platform and PCA-SVM algorithms for rapid beef meat freshness prediction
325 and identification. *Computers and Electronics in Agriculture*, 152, 385-392.

326

327 Ben Hamed, K., Zorrig, W., Hichem Hamzaoui, A. (2016). Electrical impedance spectroscopy: A
328 tool to investigate the responses of one halophyte to different growth and stress conditions.
329 *Computers and Electronics in Agriculture*, 123, 376-383.

330

331 Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gomez-Caravaca, A. M., Segura-Carretero, A.,
332 Fernandez-Gutierrez, A., Lercker, G. (2007). Phenolic molecules in virgin olive oils: a survey of
333 their sensory properties, health effects, antioxidant activity and analytical methods. An overview of
334 the last decade. *Molecules*, 12, 1679-1719.

335

336 Bera, T. K. (2014). Bioelectrical impedance methods for noninvasive health monitoring: a review.
337 *Journal of Medical Engineering*, article ID 381251, 1-28.

338

339 Bustan, A., Kerem, Z., Yermiyahu, U., Ben-Gal, A., Lichter, A., Droby, S., Zchori-Fein, E.,
340 Orbach, D., Zipori, I., Dag, A. (2014). Preharvest circumstances leading to elevated oil acidity in
341 'Barnea' olives. *Scientia Horticulturae*, 176, 11-21.

342

343 Codex Alimentarius – CODEX STAN 210-1999 - Standard for named vegetable oils codex.
344 Adopted in 1999. Revised in 2001-2003-2009-2017. Amended in 2005-2011-2013-2015.

345

346 Commission Implementing Regulation (EU) No 2016/1227 of 27 July 2016 amending Regulation
347 (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant
348 methods of analysis.

349

350 Commission Implementing Regulation (EU) No 1348/2013 of 16 December 2013 amending
351 Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the
352 relevant methods of analysis.

353

354 Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and
355 on the relevant methods of analysis.

356

357 Grossi, M., Lanzoni, M., Lazzarini, R., Riccò, B. (2012a). Automatic ice-cream characterization by
358 impedance measurements for optimal machine setting. *Measurement*, 45, 1747-1754.

359

360 Grossi, M., Lanzoni, M., Lazzarini, R., Riccò, B. (2012b). Linear non-iterative sinusoidal fitting
361 algorithm for microbial impedance biosensor. *Sensors & Transducers Journal*, 137 (2), 235-244.

362

363 Grossi, M., Di Lecce, G., Gallina Toschi, T., Riccò, B. (2014a). A novel electrochemical method
364 for olive oil acidity determination. *Microelectronics Journal*, 45 (12), 1701-1707.

365

366 Grossi, M., Di Lecce, G., Gallina Toschi, T., Riccò, B. (2014b). Fast and accurate determination of
367 olive oil acidity by electrochemical impedance spectroscopy. *IEEE Sensors Journal*, 14 (9), 2947-
368 2954.

369

370 Grossi, M., Di Lecce, G., Arru, M., Gallina Toschi, T., Riccò, B. (2015). An opto-electronic system
371 for in-situ determination of peroxide value and total phenol content in olive oil. *Journal of Food*
372 *Engineering*, 146, 1-7.

373

374 Grossi, M., Riccò, B. (2017a). Electrical impedance spectroscopy (EIS) for biological analysis and
375 food characterization: a review. *Journal of Sensors and Sensor Systems*, 6, 303-325.

376

377 Grossi, M., Riccò, B. (2017b). An automatic titration system for oil concentration measurement in
378 metalworking fluids. *Measurement*, 97, 8-14.

379

380 Harker, F. R., Maindonald, J. H. (1994). Ripening of nectarine fruit. *Plant Physiology*, 106, 165-
381 171.

382

383 Hussin, M. H., Rahim, A. A., Nasir, M., Ibrahim, M., Brosse, N. (2016). The capability of
384 ultrafiltrated alkaline and organosolv oil palm (*Elais guineensis*) fronds lignin as green corrosion
385 inhibitor for mild steel in 0.5 M HCl solution. *Measurement*, 78, 90-103.

386

387 Inajeros-García, A. M., Gómez-Alonso, S., Fregapane, G., Salvador, M. D. (2013). Evaluation of
388 minor components, sensory characteristics and quality of virgin olive oil by near infrared (NIR)
389 spectroscopy. *Food Research International*, 50, 250-258.

390

391 Khalil, S. F., Mohktar, M. S., Ibrahim, F. (2014). The theory and fundamentals of bioimpedance
392 analysis in clinical status monitoring and diagnosis of diseases. *Sensors*, 14, 10895-10928.

393

394 Lin, C. M., Chen, L. H., Chen, T. M. (2012). The development and application of an electrical
395 impedance spectroscopy measurement system for plant tissue. *Computers and Electronics in*
396 *Agriculture*, 82, 96-99.

397

398 Ouji, N. B., Bakas, I., Istamboulié, G., Ait-Ichou, I., Ait-Addi, E., Rouillon, R., Noguer, T. (2014).
399 A simple colorimetric enzymatic-assay, based on immobilization of acetylcholinesterase by
400 adsorption, for sensitive detection of organophosphorus insecticides in olive oil. *Food Control*, 46,
401 75-80.

402

403 Ragni, L., Iaccheri, E., Cevoli, C., Berardinelli, A., Bendini, A., Gallina Toschi, T. (2013). A
404 capacitive technique to assess water content in extra virgin olive oils. *Journal of Food Engineering*,
405 116 (1), 246-252.

406

407 Sture, O., Ruud Oye, E., Skavhaug, A., Mathiassen, J. R. (2016). A 3D machine vision system for
408 quality grading of Atlantic salmon. *Computers and Electronics in Agriculture*, 123, 142-148.

409

410 Tena, N., Wang, S. C., Aparicio-Ruiz, R., García-González, D. L., Aparicio R. (2015). In-depth
 411 assessment of analytical methods for olive oil purity, safety, and quality characterization. *Journal of*
 412 *Agricultural and Food Chemistry*, 63, 4509–4526.

413

414 Thompson, M., Ellison, S. L., Wood, R. (2002). Harmonized guidelines for single-laboratory
 415 validation of methods of analysis (IUPAC Technical Report). *Pure and Applied Chemistry*, 74 (5),
 416 835-855.

417

418 Tur'yan, Y. I., Berezin, O. Y., Kuselman, I., Shenhar, A. (1996). pH-metric determination of acid
 419 values in vegetable oils without titration. *Journal of the American Oil Chemists' Society*, 73 (3),
 420 295-301.

421

422 Valli, E., Bendini, A., Berardinelli, A., Ragni, L., Riccò, B., Grossi, M., Gallina Toschi, T. (2016).
 423 Rapid and innovative instrumental approaches for quality and authenticity of olive oils. *European*
 424 *Journal of Lipid Science and Technology*, 118, 1601-1619.

425

426 Veneziani, G., Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B., Servili, M. (2018a).
 427 Characterization of phenolic and volatile composition of extra virgin olive oil extracted from six
 428 Italian cultivars using a cooling treatment of olive paste. *LWT – Food Science and Technology*, 87,
 429 523-528.

430

431 Veneziani, G., Esposto, S., Minnocci, A., Taticchi, A., Urbani, S., Selvaggini, R., Sordini, B.,
 432 Sebastiani, L., Servili, M. (2018b). Compositional differences between veiled and filtered virgin
 433 olive oils during a simulated shelf life. *LWT – Food Science and Technology*, 94, 87-95.

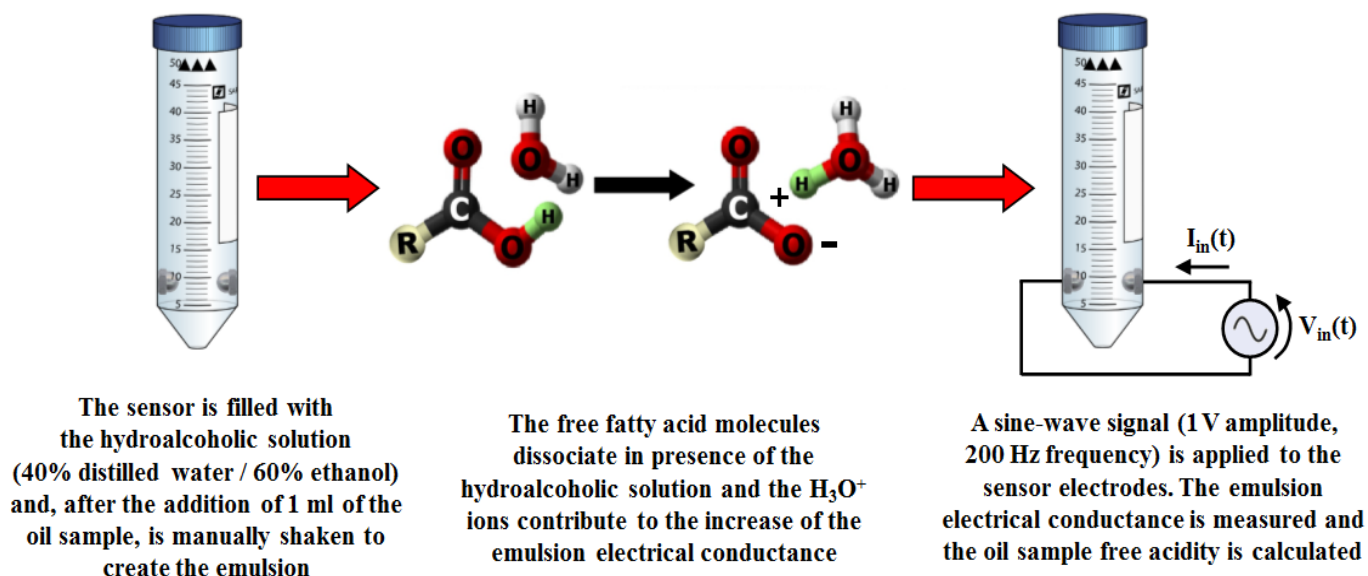
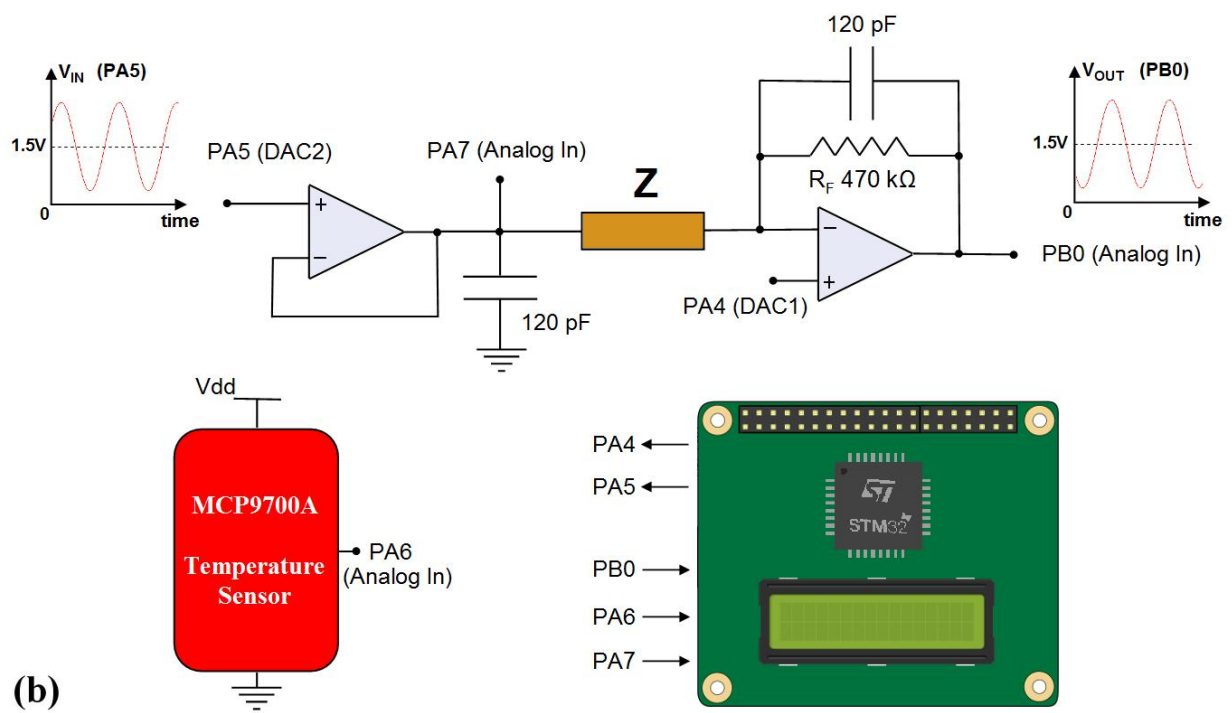
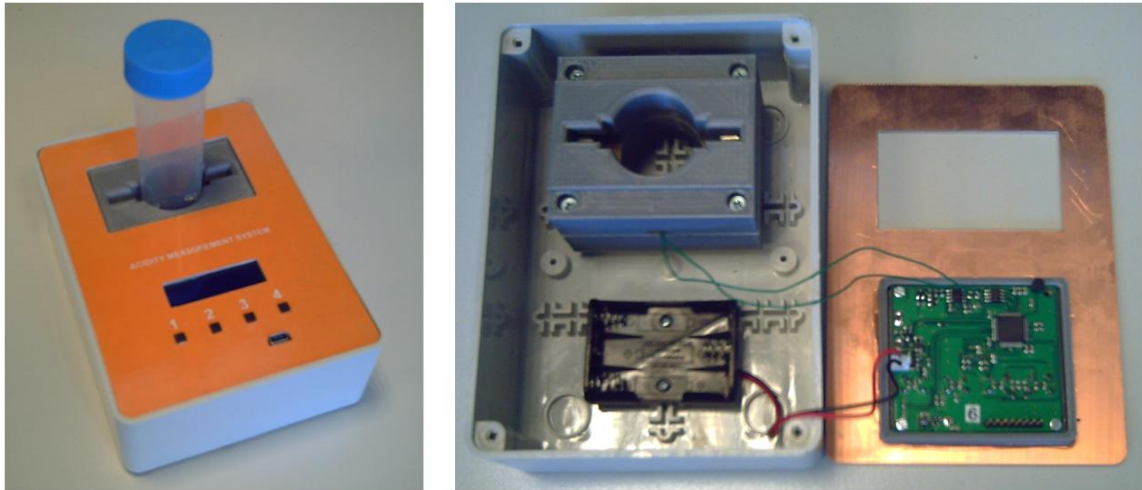


Figure 1

(a)



(b)

Figure 2

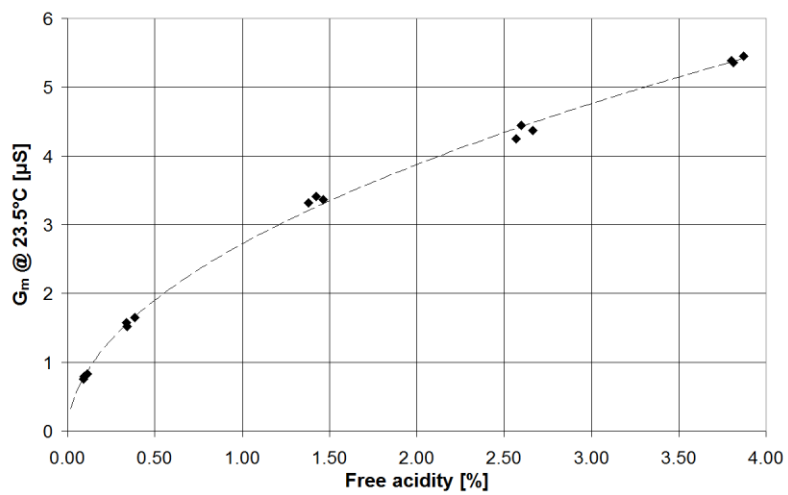


Figure 3

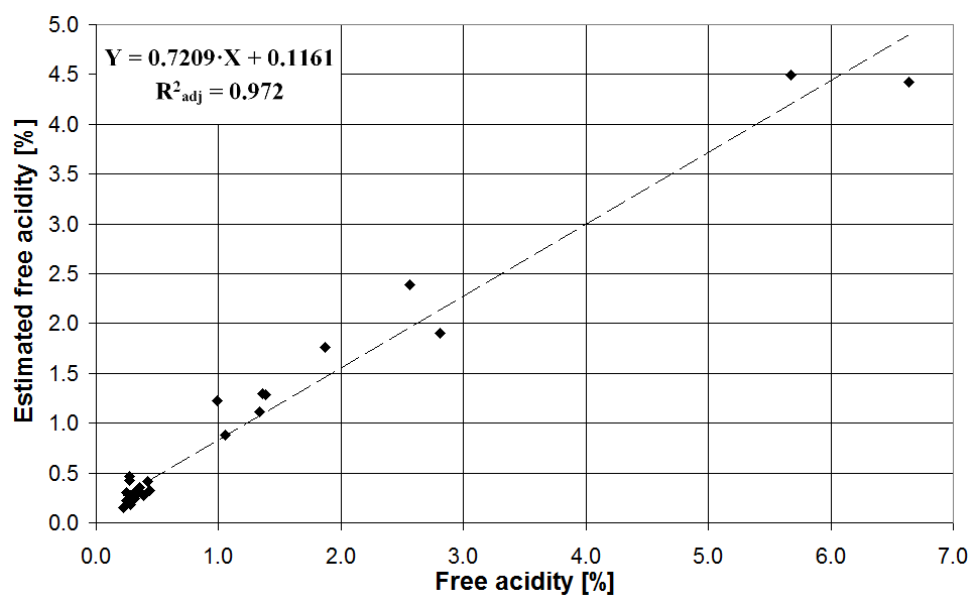


Figure 4

444 **Figure captions**

445 **Figure 1.** Scheme of the working principle of the proposed electronic system for the determination
446 of free acidity in virgin olive oil (Grossi, & Riccò, 2017a).

447 **Figure 2.** Pictures of the outside and inside of the electronic system and the sensor for olive oil free
448 acidity analysis (a); schematic of the system hardware and of its electronical functioning (b).

449 **Figure 3.** Measured electrical conductance (G_m) at 23.5°C *vs* free acidity for the sunflower oil
450 calibration set.

451 **Figure 4.** Scatter plot of the estimated free acidity *vs* the free acidity measured by titration for a set
452 of 30 olive oil samples.

453 **Table 1**

454 Codes and description of the samples used for the calibration of the system, data of free acidity
 455 determined by the official method and conductance measured with the portable system. The values
 456 are mean of three replicates.

Sample code	Sample description	Free acidity (% oleic acid)	Conductance (μS)
A	Refined sunflower oil (without addition of oleic acid)	0.10 ± 0.01	0.79 ± 0.03
B	Refined sunflower oil + 0.25% oleic acid	0.36 ± 0.03	1.58 ± 0.06
C	Refined sunflower oil + 1.25% oleic acid	1.42 ± 0.04	3.36 ± 0.04
D	Refined sunflower oil + 2.50% oleic acid	2.61 ± 0.05	4.35 ± 0.10
E	Refined sunflower oil + 3.75% oleic acid	3.83 ± 0.04	5.40 ± 0.05

457

458 **Table 2.** Values of free acidity for all samples measured by the portable system and the official
459 method. Results are expressed as % of oleic acid.

Sample code	Free acidity (portable system)	Free acidity (official method)
EVOO_1	0.18	0.25
EVOO_2	0.31	0.34
EVOO_3	0.25	0.28
EVOO_4	0.43	0.27
EVOO_5	0.30	0.25
EVOO_6	0.22	0.25
EVOO_7	0.18	0.25
EVOO_8	0.29	0.37
EVOO_9	0.29	0.34
EVOO_10	0.47	0.27
EVOO_11	0.32	0.33
EVOO_12	0.41	0.42
EVOO_13	0.24	0.28
EVOO_14	0.27	0.28
EVOO_15	0.32	0.44
EVOO_16	0.35	0.35
EVOO_17	0.18	0.28
EVOO_18	0.15	0.22
EVOO_19	0.27	0.39
EVOO_20	0.24	0.31
VOO_1	1.30	1.36
VOO_2	1.29	1.38
VOO_3	0.88	1.05
VOO_4	1.11	1.33
VOO_5	1.22	0.99
VOO_6	1.76	1.87
VOO_7	1.90	2.81
LOO_1	2.39	2.56
LOO_2	4.42	6.64
LOO_3	4.49	5.67

460

Appendix A

According to the working principle of the portable system, a 50 mL polypropylene tube (Falcon) vial modified to feature a couple of stainless-steel electrodes to measure the emulsion conductance (hereafter the sensor) is filled with 15 mL of hydro-alcoholic solution (40% distilled water/60% ethanol), then 1 mL of the olive oil sample is added and all is stirred to create an emulsion.

In presence of the hydroalcoholic solution, the free fatty acid molecule RCOOH , where R is the hydrocarbon chain, dissociates in the ionic compounds H_3O^+ and RCO_2^- that contribute to the increase of the emulsion electrical conductance. In the end, the higher the free fatty acid molecules concentration, the higher ions concentration and the higher the electrical conductance.

The emulsion electrical conductance is measured by Electrical Impedance Spectroscopy (EIS).

In the proposed approach the sample under investigation is stimulated with a sine-wave voltage signal $V_{in}(t)$:

$$V_{in}(t) = V_{M,in} \times \sin(2\pi ft) \quad (\text{A1})$$

and the current $I_{in}(t)$ through the sample is measured:

$$I_{in}(t) = I_{M,in} \times \sin(2\pi ft + \varphi) \quad (\text{A2})$$

where $V_{M,in}$ and $I_{M,in}$ are the amplitudes of the corresponding signals, f is the frequency of the test signal and φ is the phase difference between $I_{in}(t)$ and $V_{in}(t)$.

The sample electrical admittance is then expressed as:

$$Y = \frac{I_{in}(j2\pi f)}{V_{in}(j2\pi f)} = \frac{I_{M,in}}{V_{M,in}} \times (\cos \phi + j \times \sin \phi) = \text{Re}(Y) + j \times \text{Im}(Y) \quad (\text{A3})$$

The emulsion in direct contact with the electrodes can be modelled as the parallel of an electrical conductance (accounting for the conductance of the emulsion) and a capacitance (accounting for the emulsion dielectric properties): while the emulsion conductance (that dominates at low frequency) is affected by the sample acidity due to the variation of the ions concentration, the dielectric properties are almost independent. Thus, the emulsion electrical conductance G_m can be estimated with the real component of the emulsion admittance $\text{Re}(Y) = |Y| \cdot \cos(\varphi)$ where $|Y|$ is the admittance modulus.

In Figure A1 (a) the admittance modulus (measured with the commercial LCR meter Agilent E4980A) is plotted vs. the frequency of the applied test signal for different samples featuring different acidity in the frequency range 20 Hz – 2 MHz. As can be seen, $|Y|$ increases with sample acidity for test signals with a frequency lower than 20 kHz while it is almost independent on sample acidity for higher frequencies. This is the reason why the designed electronic system measures G_m with a single frequency measurement at 200 Hz.

The relation between G_m and the free acidity is non-linear and it can be modelled with the function:

$$G_m = \alpha + \beta \times \sqrt{FA} + \gamma \times \sqrt[4]{FA^3} \quad (A4)$$

where α , β and γ are empirical parameters that must be determined by a suitable calibration procedure and are also function of the calibration temperature.

In Figure A1 (b) the qualitative plot of G_m vs FA is shown: as can be seen, the non-linear function results in better accuracy for the estimated free acidity for lower acidity levels. This has been taken in account by using a suitable amount of sample to create the emulsion to obtain good accuracy in the acidity range of interest.

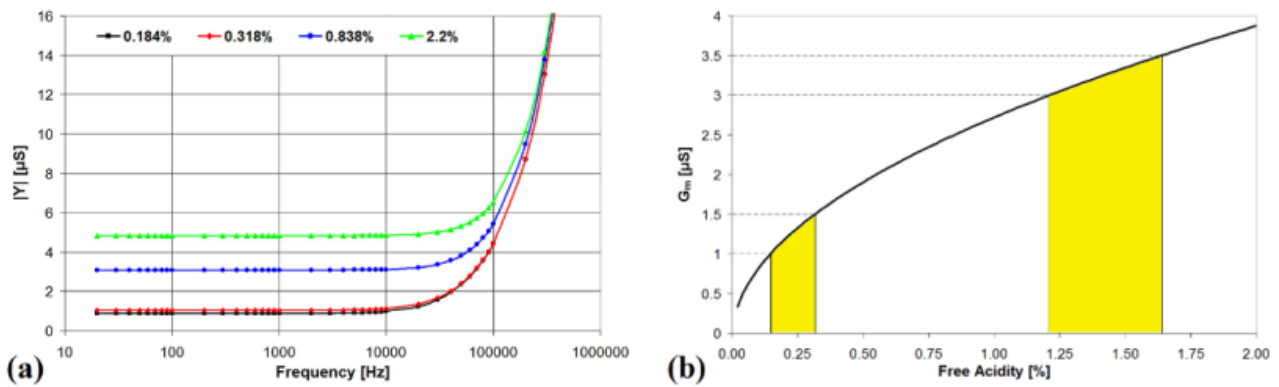


Figure A1 (a) admittance modulus plotted vs frequency for olive oil samples featuring different free acidity; (b) qualitative plot of the electrical conductance as function of sample acidity.

However, eq. A4 needs a computation intensive iterative algorithm to extract the estimated acidity from the measured value of G_m as well as manual input of starting point to avoid failing in algorithm convergence. Thus, a simpler model has been chosen (obtained by neglecting the molar conductivity dependence on the H_3O^+ ions concentration) that is much more suitable to be implemented in a low-cost microcontroller and can be described by the following function:

$$G_m = \alpha + \beta \times \sqrt{FA} \quad (A5)$$

The two models of eq. A4 and A5 have been tested with the data set of Grossi et al. (Grossi et al., 2014b) and the results have shown how the accuracy in estimating free acidity is only marginal higher for model A4 than model A5.

The sample free acidity can thus be estimated from the measured electrical conductance of the emulsion with the following formula:

$$FA = \left(\frac{G_m - \alpha}{\beta} \right)^2 \quad (A6)$$

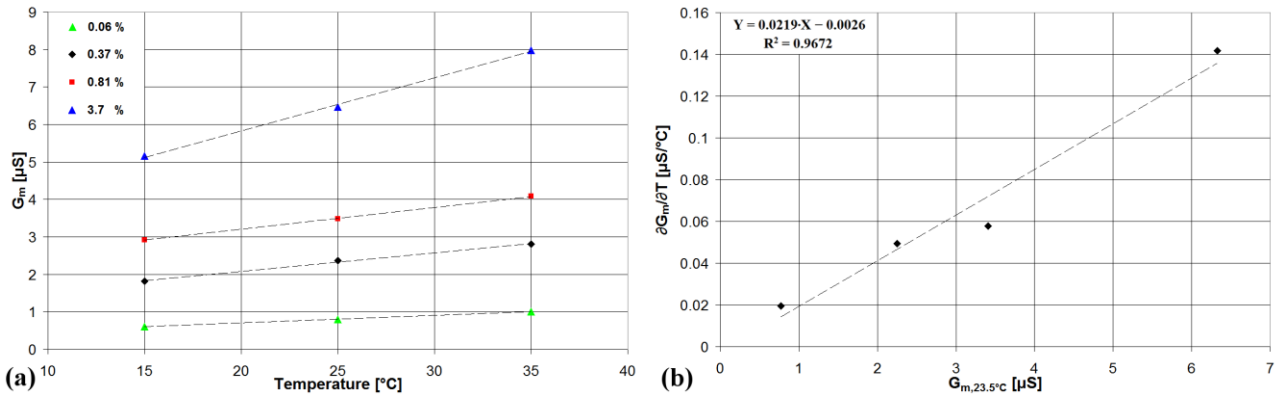
Appendix B

520 The oil sample free acidity can be estimated by measuring the emulsion electrical conductance at
 521 the temperature of calibration and then calculating the free acidity using equation A6. However,
 522 since the system must be operated “in the field” and the environmental temperature is not a
 523 parameter under control, there is the need to investigate how the emulsion conductance varies with
 524 the temperature so that the oil free acidity can be estimated by the measure of the emulsion
 525 conductance and the temperature.

526 Four different olive oil samples featuring different free acidity values (0.06% sample A, 0.37%
 527 sample B, 0.81% sample C and 3.7% sample D) were tested inside a Binder APT KB 53 thermal
 528 incubator for different temperatures between 15°C and 35°C.

529 In Figure B1 (a) the measured emulsion conductance is plotted vs the incubation temperature for
 530 each sample. In all cases the G_m is a linear function of the temperature with determination
 531 coefficients $R^2 > 0.99$. The calculated linear regression lines allow to determine the conductance
 532 variation with temperature (i.e. $\partial G_m / \partial T$) for all samples: 0.0196 for sample A, 0.0495 for sample B,
 533 0.0579 for sample C and 0.1417 for sample D. $\partial G_m / \partial T$ is thus found to increase with the sample
 534 free acidity. Since the sample free acidity is also a function of the emulsion electrical conductance,
 535 the relation between $\partial G_m / \partial T$ and $G_{m,23.5^\circ C}$ has been plotted in Figure B1 (b).

536



537

538 **Figure B1** (a) measured emulsion conductance vs the incubation temperature for olive oil samples
 539 featuring different free acidity; (b) $\partial G_m / \partial T$ plotted vs the electrical conductance at 23.5 $^\circ C$.

540

541 As can be seen, a linear relation gives a good approximation ($R^2 = 0.9672$) of the function between
 542 $\partial G_m / \partial T$ and $G_{m,23.5^\circ C}$ thus:

$$543 \quad \frac{\partial G_m}{\partial T} = 0.0219 \times G_{m,23.5^\circ C} - 0.0026 \quad (B1)$$

544 where $\partial G_m / \partial T$ and $G_{m,23.5^\circ C}$ are expressed as $\mu S / ^\circ C$ and μS respectively. The emulsion electrical
 545 conductance at the environmental temperature T can thus be expressed as:

$$546 \quad G_{m,T} = G_{m,23.5^\circ C} + \frac{\partial G_m}{\partial T} \times (T - 23.5) \quad (B2)$$

547 and

548
$$G_{m,T} = G_{m,23.5^{\circ}C} + (0.0219 \times G_{m,23.5^{\circ}C} - 0.0026) \times (T - 23.5) \quad (B3)$$

549 The emulsion electrical conductance at $T_{calib} = 23.5^{\circ}C$ can thus be estimated from the electrical
550 conductance at temperature T and the measured value of T using the following formula:

551
$$G_{m,23.5^{\circ}C} = \frac{G_{m,T} + 0.0026 \times (T - 23.5)}{1 + 0.0219 \times (T - 23.5)} \quad (B4)$$

552 Thus, by measuring $G_{m,T}$ and T , the value of $G_{m,23.5^{\circ}C}$ can be calculated using equation B4 and, from
553 this value, the sample acidity can be estimated using equation A6.

Video

[Click here to download Video: acidity_measure_system_english.wmv](#)